

WHAT IS CLAIMED IS:

1. A method for obtaining a composition having immune stimulating activity or anti-tumor activity from *Withania Somnifera* comprising:
 - 5 (a) contacting *Withania Somnifera* plant or plant part with a first medium polar solvent to produce a particulate suspension;
 - (b) clarifying the particulate suspension to produce a clarified first solution and a first residue;
 - (c) evaporating the solvent from the first clarified solution to produce a fraction, denoted fraction A;
 - 10 (d) resuspending the first residue in a second polar solvent thereby producing a second solution and a second residue;
 - (e) clarifying the second solution to produce a second clarified solution;
 - (f) evaporating the second polar solvent from the second clarified solution to produce a fraction, denoted fraction B;
 - 15 (g) resuspending the second residue in a third solvent more polar than the second polar solvent thereby producing a third solution and a third residue;
 - (h) clarifying the third solution to produce a third clarified solution;
 - (i) evaporating the third solvent from the third clarified solution to produce a fraction, denoted fraction C;
 - 20 (j) combining fractions A, B and C to produce an extract;
 - (k) resuspending the extract in a solution to produce a fourth alkaline solution; and
 - (l) fractionating the fourth solution with a non polar solvent and removing the solvent to produce a composition having immune stimulating activity or anti-tumor activity.
- 25 2. The method of claim 1, wherein fractions A, B and C are combined in approximately equal proportions by mass.
3. The method of claim 1, wherein fractions A, B and C are combined in unequal proportions by mass.

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4. The method of claim 1, wherein the first residue is resuspended in a solvent having about 50% ethanol or about 40 to 60% isopropyl alcohol.

5. The method of claim 1, wherein the second residue is resuspended in water.

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6. The method of claim 1, wherein step 1) removes one or more alkaloids.

7. The method of claim 1, wherein step 1) removes one or more withanolides.

10 8. The method of claim 1, wherein step 1) comprises fractionating the extract with methylene chloride, diethyl ether or chloroform.

9. The method of claim 1, wherein the plant part comprises a root.

10 10. The method of claim 1, wherein the first medium polar solvent comprises acetone, tetrahydrofuran or ethylacetate.

11. The method of claim 1, wherein the second solvent comprises a mixture of water and isopropyl alcohol (IPA).

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12. The method of claim 1, wherein the third solvent comprises water.

13. The method of claim 1, wherein the first or second solvent comprises an alcoholic organic solvent.

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14. The method of claim 1, wherein step a) comprises soaking the plant or plant part in the first solvent for at least about 2 hours.

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15. A composition having immune stimulating activity or anti-tumor activity produced by the method of claim 1.

16. The composition of claim 15, wherein the composition is characterized as having a TLC profile the same as a profile set forth in Figures 1A, 2A or 3A, the profile obtained with a hexane:methylene chloride:methanol mobile phase in about a 20:30:2 ratio.

5 17. The composition of claim 15, wherein the composition is characterized as having an HPLC profile substantially the same as a profile set forth in Figures 1B, 2B or 3B, said profile obtained using a reverse-phase C-18 column at a flow rate of about 1.2 ml/min with a mobile phase of methanol:water in a ratio of about 60:40.

10 18. A composition having immune stimulating activity or anti-tumor activity, said composition comprising any one of the molecules in peaks 1 to 5 or 7 to 9 set forth in Figures 1A, 2A or 3A, or a combination of two or more molecules in said peaks.

15 19. A composition obtained from *Withania Somnifera* characterized as:

- (a) having immune stimulating activity or anti-tumor activity;
- (b) soluble in water;
- (c) substantially free of alkaloids; and
- (d) having at least one glycowithanolide.

20 20. The composition of claim 19, further characterized as having a TLC profile substantially the same as a profile set forth in Figures 1A, 2A or 3A, the profile obtained with a hexane:methylene chloride:methanol mobile phase in about a 20:30:2 ratio.

25 21. The composition of claim 19, further characterized as having an HPLC profile substantially the same as a profile set forth in Figures 1B, 2B or 3B, said profile obtained using a reverse-phase C-18 column at a flow rate of about 1.2 ml/min with a mobile phase of methanol:water in a ratio of about 60:40.

22. The composition of claim 19, further characterized as substantially free of withanolides.

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23. The composition of claim 19, further characterized as having a glycowithanolide content from about 0.5 to 1.6% by weight.

24. The composition of claim 19, wherein the glycowithanolide comprises sitoindoside IX.

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25. The composition of claim 19, wherein the glycowithanolide comprises sitoindoside X.

26. The composition of claim 19, wherein the glycowithanolide comprises a mixture of sitoindoside IX and sitoindoside X.

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27. The composition of claim 19, wherein the glycowithanolide comprises a mixture of sitoindoside IX, sitoindoside X and one or more glycowithanolides distinct from sitoindoside IX and sitoindoside X.

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28. The composition of claim 19, wherein the glycowithanolide comprises a mixture of sitoindoside IX, sitoindoside X and two or more glycowithanolides distinct from sitoindoside IX and sitoindoside X.

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29. The composition of claim 19, wherein the glycowithanolide comprises a mixture of sitoindoside IX, sitoindoside X and three or more glycowithanolides distinct from sitoindoside IX and sitoindoside X.

30. The composition of claim 19, further characterized as having one or more of the following by mass:

25 (a) about 35-75% protein content;

(b) about 0.5 to about 5% glycowithanolide(s);

(c) about 3 to about 10% ash; and

(d) about 30 to about 60% carbohydrate.

31. The composition of claim 19, wherein administering about 50 mg/kg subject mass of the composition to a Balb-c mouse increases by about 20% or more the number of white blood cells in the Balb-c mouse.

5 32. The composition of claim 31, wherein the subject has less than normal numbers of white blood cells.

10 33. The composition of claim 19, wherein administering about 100 mg/kg subject mass of the composition to a Balb-c mouse increases by about 20% or more the number of white blood cells in the Balb-c mouse.

34. The composition of claim 33, wherein the subject has less than normal numbers of white blood cells.

15 35. A pharmaceutical formulation comprising the composition of claim 17, and a pharmaceutically acceptable carrier.

36. The formulation of claim 35, further comprising a drug.

20 37. The formulation of claim 35, wherein the drug increases white blood cell numbers in a subject.

38. The formulation of claim 35, wherein the drug has immunosuppressing activity in a subject.

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39. The formulation of claim 35, wherein the drug inhibits cell cycle progression.

40. The formulation of claim 35, wherein the drug inhibits cell proliferation.

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The formulation of claim 35, wherein the drug comprises an anti-tumor drug.

42. The formulation of claim 41, wherein the anti-tumor drug inhibits nucleic acid or protein synthesis.

43. The formulation of claim 38, wherein the drug comprises a steroid glycoside.

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44. The formulation of claim 35, wherein the drug comprises an alkylating agent, an anti-metabolite, a plant alkaloid, a plant extract, an antibiotic, a nitrosourea, a hormone, a nucleoside analogue, or a nucleotide analogue.

10 45. The formulation of claim 35, wherein the drug is selected from cyclophosphamide, azathioprine, cyclosporin A, prednisolone, melphalan, chlorambucil, mechlorethamine, busulphan, methotrexate, 6-mercaptopurine, thioguanine, 5-fluorouracil, cytosine arabinoside, AZT, 5-AZC, taxol, vinblastine, vincristine, doxorubicin, bleomycin, actinomycin D, mithramycin, mitomycin C, carmustine, lomustine, semustine, streptozotocin, hydroxyurea, cisplatin, mitotane, procarbazine, dacarbazine or dibromomannitol.

46. The formulation of claim 35, wherein the excipient is suitable for injection or infusion.

20 47. The formulation of claim 35, wherein the formulation comprises a pill, granules, crystals, a capsule, a syrup, a suspension, an elixir or an injectable.

25 48. A kit comprising the pharmaceutical formulation of claim 35, and instructions for use in stimulating an immune response or in potentiating anti-cell proliferative activity of an anti-cell proliferative therapy

49. A method for increasing the number of white blood cells in a subject comprising administering to a subject an amount of the composition of claim 17 effective to increase the number of white blood cells in the subject.

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50. The method of claim 49, wherein the white blood cells are selected from monocytes, macrophages, natural killer cells, dendritic cells, granulocytes, basophils and eosinophils.

5 51. The method of claim 49, wherein the subject has less than normal numbers of white blood cells.

52. The method of claim 49, wherein the subject has been, is currently undergoing or will be undergoing an immunosuppressive therapy.

10 53. The method of claim 49, wherein the subject has been, is currently undergoing or will be undergoing a cancer therapy.

15 54. The method of claim 53, wherein the cancer therapy comprises administration of radiation or a radioisotope.

55. The method of claim 53, wherein the subject has asthma, rheumatoid arthritis, or psoriasis.

20 56. A method for reducing immunosuppression in a subject comprising administering to an immunosuppressed subject, or a subject at risk of immunosuppression, an amount of the composition of claim 19 effective to reduce immunosuppression in the subject.

57. The method of claims 49 or 56, wherein the subject is treated prophylactically.

25 58. The method of claims 49 or 56, wherein the amount administered comprises a dose of about 10 to 50, 50 to 100, or 100 to 200 mg composition/kg subject mass.

59. The method of claims 49 or 56, wherein the composition is administered in multiple doses.

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60. The method of claims 49 or 56, wherein the composition is administered via injection, gradual perfusion or intubation.

61. The method of claims 49 or 56, wherein the composition is administered orally.

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62. The method of claims 49 or 56, wherein the composition is administered prior to, contemporaneously with, or after administering a drug.

10 63. The method of claim 62, wherein the drug stimulates or suppresses an immune response.

15 64. A method for increasing activity of an anti-tumor drug comprising administering to a subject treated with an anti-tumor drug the composition of claim 19 prior to, contemporaneously with or after administering the anti-tumor drug to the subject.

20 65. The method of claim 64, wherein the anti-tumor drug comprises a radioisotope, an alkylating agent, an anti-metabolite, a plant alkaloid, a plant extract, an antibiotic, a nitrosourea, a hormone, a nucleoside analogue, or a nucleotide analogue.

66. The method of claim 64, wherein the amount administered comprises a dose of about 10 to 50, 50 to 100, or 100 to 200 mg composition/kg subject mass.

25 67. The method of claim 64, wherein the subject is at risk of, presently has or previously had cancer.

68. The method of claim 67, wherein the cancer comprises a solid or liquid tumor.

26 69. The method of claim 68, wherein the cancer comprises a breast, brain, head or neck, eye, nasopharynx, lung, liver, pancreas, kidney, esophagus, stomach, small or large intestine, bladder, rectal, prostate, testicular, ovarian, uterine, bone, muscle or skin tumor.

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70. The method of claim 68, wherein the solid tumor comprises a fibrosarcoma, lymphosarcoma, liposarcoma or osteosarcoma.
71. The method of claim 68, wherein the liquid tumor comprises a lymphoma, leukemia or myeloma.
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72. The method of claim 64, wherein the composition is administered at intermittent frequencies or variable dosages.